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Impacts and Pathways of Mine Contaminants to Bull Trout (*Salvelinus confluentus*) in an Idaho Watershed

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Abstract Metals contamination from mining activities is a persistent problem affecting aquatic ecosystems throughout mining districts in the western USA. The Gold Creek drainage in northern Idaho has a history of mining within its headwaters and contains elevated sediment concentrations of As, Cd, Cu, Pb, and Zn. To determine system-wide impacts of increased metals, we measured concentrations of metals in water, sediment, and benthic macroinvertebrate tissues and related them to whole-body fish tissues and histopathological alterations in native salmonids. Water concentrations were higher than those in reference areas, but were below water quality criteria for protection of aquatic biota for most of the study area. Sediment and benthic macroinvertebrate tissue concentrations for all metals were significantly higher at all sites compared with the reference site. Fish tissues were significantly higher for all metals below mine sites compared with the reference site, but only Cd and Pb were higher in fish tissues in the furthest downstream reach in the Gold Creek Delta. Metals concentrations in benthic macroinvertebrate tissues and fish tissues were strongly correlated, suggesting a transfer of metals through a dietary pathway. The concentrations within sediments and biota were similar to those reported in other studies in which adverse effects to salmonids occurred. We observed histopathological changes in livers of bull trout, including inflammation, necrosis, and pleomorphism. Our study is consistent with other work in which sediment-driven exposure can transfer up the food chain and may cause adverse impacts to higher organisms.

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Bull trout (*Salvelinus confluentus*) historically ranged from the McCloud River in California to southern Alaska (Cavender 1978). Due to declines throughout their range, bull trout were listed as threatened under the US Endangered Species Act in 1998 (63 FR 31647). Factors that may have contributed to population decline and fragmentation include loss of habitat, competition with exotic species, and hydropower operations (Goetz 1989; 63 FR 31647). Bull trout require headwater tributaries with cold temperatures, good water quality, and coarse-bottom substrates for spawning and rearing (Thurow and Schill 1996). Juvenile bull trout depend on hard-cobble bottoms and are closely associated with substrates for feeding and cover (Pratt 1992).

From the mid 1800s through the early 1970s, extensive hard-rock mining occurred throughout the western USA. During this period, mining operations commonly disposed of waste rock and tailings into nearby watersheds, leaving extensive metals contamination in water and sediments. Arsenic (As) is a contaminant of particular concern commonly associated with hard-rock mining wastes throughout the inland northwest USA.

In some cases mine-related contamination may be a significant factor in bull trout population declines. Previously, only laboratory studies investigating aqueous metals exposure to bull trout have been undertaken (Hansen et al. 2001, 2002). However, even when contaminant concentrations in

water are below water quality criteria, impacts from sediment exposure pathways may still occur (Bergman and Dorward-King 1997). To our knowledge, metals exposure to bull trout through a dietary pathway under field conditions has not been evaluated.

Macroinvertebrates accumulate metals through feeding strategies and their close association with sediments (Farag et al. 1999). Benthic macroinvertebrates are the primary forage of young-of-year salmonids within mountain streams, and sensitive young-of-year salmonids may receive high doses of contaminants transferred through their trophic interactions (Rainbow 1996). Other studies have used benthic macroinvertebrates from metals-contaminated rivers in laboratory dietary exposure studies and demonstrated adverse effects from the consumption of metals-contaminated invertebrates (Farag et al. 1999; Woodward et al. 1994). Arsenic, for example, is thought to be primarily accumulated in salmonids through the dietary route, but to our knowledge, the effect of As on salmonids is poorly understood (Ghosh et al. 2006; Sorenson 1991).

Metals burdens in fish tissue are a common measure of exposure and may be correlated to histopathological effects (Hansen et al. 2004). Histological change can occur in various organs as result of chronic stressors and can serve as useful biomarker of exposure to contaminants (Bernet et al. 1999). Dietary As has been shown in several laboratory studies to be associated with characteristic gallbladder lesions in salmonids (Cockell and Hilton 1988; Cockell et al. 1991, 1992; Hansen et al. 2004; McGeachy and Dixon 1990). Degenerative changes observed in the gallbladder included inflammation, edema, sloughing of the lamina epithelia, hemorrhages, and cellular debris and bleeding within the lumen (Cockell and Bettger 1993). These changes disrupt digestive mechanisms in young fish and ultimately reduce fitness and survival. Researchers have suggested that gallbladder pathology may be a useful indicator of chronic As exposure in salmonids for field investigations (Pedlar et al. 2001).

The objectives of this study are to: (1) determine the spatial extent of contamination in water and sediments within the study area, (2) relate water and sediment concentrations with benthic macroinvertebrate metals residues to evaluate metals dietary exposure pathways, and (3) relate fish tissue metals burdens to histopathological alterations to determine risks to bull trout.

Materials and Methods

Study Site

Gold Creek is a tributary to Lake Pend Oreille in northern Idaho (Fig. 1). Gold Creek is the second most productive stream for spawning and rearing of bull trout within the Lake Pend Oreille Basin in terms of redd counts and juvenile density measurements (Downs et al. 2003; Rieman and Myers 1997). Consequently, this stream is considered ecologically important to conserve and recover healthy populations of bull trout within the Lake Pend Oreille Basin. However, Gold Creek is also highly contaminated with mine waste. Several abandoned mine sites are located in the headwaters of this drainage, and contamination may pose a threat to aquatic biota. Arsenic concentrations in mine tailings were 8,500 mg As/kg below Lakeview Mine in Chloride Gulch, and concentrations up to 1,200 mg As/kg have been measured as far as 2.5 km downstream of the mine site (Weston 2002). The Gold Creek Delta in Lake Pend Oreille contained sediment metals concentrations up to 630 mg As/kg (Weston 2002). In 2003, the US Environmental Protection Agency (USEPA) and US Forest Service (USFS) began remediation efforts at Lakeview Mine. Remediation was initiated not only to reduce public health and safety risks associated with contaminated soils

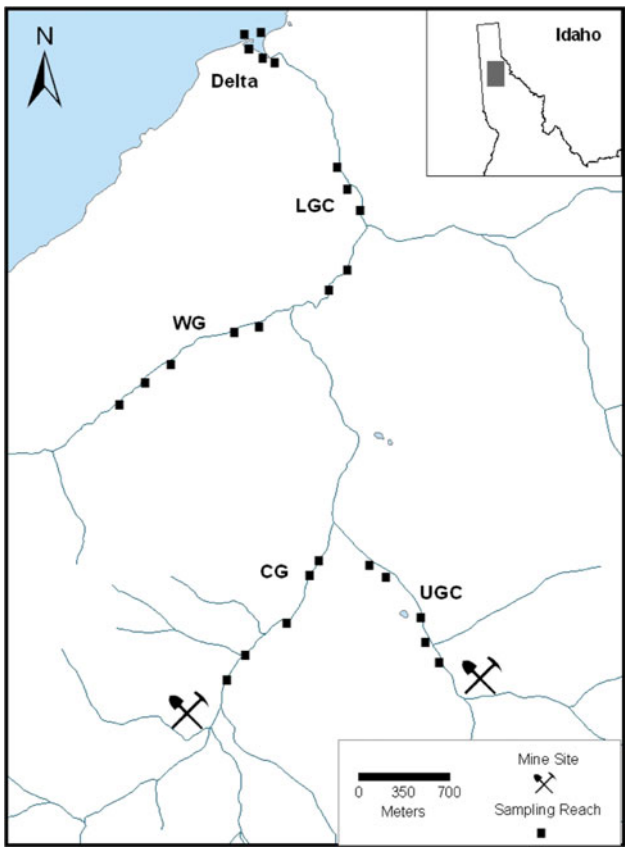

Fig. 1 Map showing the mine sites, sampling sites, and reaches within the study area in Bonner County, northern Idaho, USA. Sampling sites include Chloride Gulch (CG) below Idaho Lakeview Mine, upper Gold Creek (UGC) below Conjecture Mine, West Gold Creek (WG) the reference site, lower Gold Creek (LGC), and the Gold Creek Delta in Lake Pend Oreille (Delta)

and surface water, but to conduct an ecologically based cleanup to protect threatened bull trout within the watershed. The agencies removed 25,000 tons of surface contaminants from the mine site, with a target cleanup for nearby soils of 700 mg As/kg. It is unclear if present target cleanup levels and removal of surface contaminants will be protective of bull trout within the entire watershed. It is therefore of interest to understand and quantify how past mining contaminants and remediation is impacting this critical bull trout population within Gold Creek.

Site Selection

Gold Creek was partitioned into five sites for sampling to establish a gradient from unimpacted sites to impacted sites, and five 150-m reaches were randomly chosen within each site (Fig. 1). The five sites included: (1) lower Gold Creek (LGC) below the confluence with West Gold to the Lake Pend Oreille Delta; (2) upper Gold Creek (UGC) above the confluence with Chloride Gulch below Conjecture Mine; (3) Chloride Gulch (CG) below Lakeview Mine; (4) West Gold (WG), which has no history of mining and little timber harvest, to serve as our reference site; and (5) the Delta of Gold Creek in Lake Pend Oreille.

Water Collection

Water samples were collected from July through August of 2006 and 2007 ($N = 25$ for each year) during low flows. Each sample was filtered through a prerinsed [5% nitric acid/nanopure deionized (DI) water] 0.45-μm disc filter attached to an acid-washed 60-ml syringe. The sample was filtered into a precleaned 150-ml polypropylene sample bottle and immediately placed on ice. The samples were later ($\leq$2 h) preserved with concentrated nitric acid to pH $<$2. In addition, duplicate water samples were taken at two sites as well as blank samples of nanopure DI water. During water sampling, pH, temperature, and specific conductivity were measured with regularly calibrated YSI 85 (YSI Inc., Yellow Springs, OH) and Orion 265A (Thermo Inc. Baton Rouge, LA) instruments. To further analyze possible aqueous exposure by taking water chemistry into account, we used the Biotic Ligand Model version 2.2.3 (HydroQual Inc. Mahwah, NJ) for Cd, Cu, and Zn 50% lethal concentration (LC_{50}) predictions. Results are reported in μg/l.

Inorganic Sediment Collection

Sediments were collected from July through August of 2006 and 2007 ($N = 25$ for each year). Two sediment samples were taken in depositional zones within each 150-m reach. Samples were collected with polystyrene sediment scoops from stream reaches or a Petite Ponar sampler within the Delta. Sediments were placed in an acid-washed polypropylene bowl and thoroughly mixed, then placed into 250-ml wide-mouth polypropylene jars. Samples were later frozen until analysis. Sediment samples were digested in the laboratory in one of two ways. One sample was leached with weak acid (1 N HCl), followed by separation of the leachate by centrifugation. This weak-acid extraction method was equivalent to methods for simultaneously extracted metals (SEM), which is representative of the fraction bioavailable to biological receptors (Ingersoll 2007). The other sample was digested using strong acid extraction of (HNO_3/HCl), followed by heating, agitation, and centrifugation. The strong-acid digestion follows EPA method 3050B for estimation of recoverable metals that may become environmentally available. The sediment sample concentrations from weak-acid extractions were used for reporting and statistical comparisons. In addition, three blanks and field duplicates were also taken. Results are reported in mg/kg on dry weight basis.

Organic Sediment Collection

To determine impacts from other mining-associated contaminants, an additional sediment sample was collected from each reach in August of 2007 for analysis of chlorinated hydrocarbons and polycyclic aromatic hydrocarbons ($N = 25$). Samples were collected in depositional areas with a stainless-steel sediment scoop and thoroughly homogenized in a stainless-steel mixing bowl. Sediment material was removed and the samples were placed into clean 250-ml glass wide-mouth jars. We also collected additional blanks and field duplicates. Samples were immediately placed on ice and frozen within 8 h for preservation.

Macroinvertebrate Tissues

Benthic macroinvertebrates were collected from July to August of 2006 ($N = 25$). Sampling effort began at the downstream end of each reach and progressed upstream. All suitable habitats were sampled using a 500-μm-mesh 1-m^2 kick net. Benthic macroinvertebrates were removed from the net with 5% nitric acid-rinsed forceps. Macroinvertebrates of various taxa and sizes were collected to obtain a representative sample of bull trout diets including mayflies, stoneflies, caddisflies, dipterans, and oligochaetes. Samples were placed into clean 125-ml wide-mouth polypropylene jars and immediately placed on dry ice. Results are reported in μg/g on dry weight basis.

Fish Tissues

Whole-body fish were collected from July through August 2006 ($N = 25$). Fish were collected using a Smith-Root

LR-24 backpack electrofisher incorporating pulsed direct current (DC). Sampling began at the downstream section of each reach and progressed upstream. Juvenile salmonids were collected between 50 and 75 mm. Westslope cutthroat (*Oncorhynchus clarki lewisi*) trout were used as surrogates where bull trout were absent. We considered westslope cutthroat appropriate surrogates for bull trout due to the dietary overlap between these two juvenile salmonid species across this size range in headwater habitats (Pratt 1992). Whole-body samples were immediately placed into 125-ml wide-mouth polystyrene jars and placed on dry ice. Results are reported in μg/g on dry weight basis.

Analytical Methods

Water, sediments, and biological tissues samples were analyzed by inductively coupled plasma–mass spectrometry (ICP-MS) for As, cadmium (Cd), copper (Cu), lead (Pb), zinc (Zn), and other prevalent metals. Sediments were also analyzed for chlorinated hydrocarbons by gas chromatography, and polycyclic aromatic hydrocarbons were analyzed by mass spectrometry.

Histopathology

Fish histology samples were collected along with fish whole-body metals samples in 2006 ($N = 25$). Collection procedures follow those described above. Fish used for histopathological analysis were euthanized with 1:10 clove oil/ethanol mixture and then rinsed with ambient water. A ventral incision was made to expose the peritoneal cavity before preserving the fish in Davidson's fixative solution. The samples were sent to the US Fish and Wildlife Service Fish Health Center (Bozeman, MT, USA) for histopathological analysis. Each fish was embedded in paraffin and sectioned sagittally. Four, 5-μm-thick tissue sections were taken at different depths from each block; two were stained with hematoxylin and eosin and the other two with Giemsa (Beth MacConnell, US Fish and Wildlife Service, histopathologist, personal communication). Up to three additional hematoxylin and eosin sections were cut from each fish to obtain adequate views of gallbladder tissue. All slides were examined with no knowledge of sample site. A minimum of four tissue sections were examined for each fish.

Statistical Analyses

Metals concentrations (As, Cd, Cu, Pb, and Zn) in water, sediment, benthic macroinvertebrate tissues, and fish tissues were tested for normality and homogeneity using Anderson–Darling and F-tests. Data that failed these tests were log transformed. We then tested the data using one-way analysis of variance (ANOVA). Tests that were statistically significant were then compared using Dunnett's multiple-comparison procedure comparing all sites with the reference site. We used linear regression models to determine relationships between water, sediments, benthic macroinvertebrate, and fish tissue metals concentrations. Statistical tests were conducted using Minitab® version 15.1 (Mintab Inc. State College, PA). All tests were considered statistically significant at $p \leq 0.05$.

Results

Extent of Contamination in Water and Sediments

Dissolved metals concentrations within water (μg/l) were elevated at all sites compared with the reference site (Table 1). Chloride Gulch was significantly higher than the reference site for As ($p < 0.0001$), Cd ($p = 0.0007$), and Zn ($p = 0.001$). Upper Gold Creek was significantly higher for As ($p = 0.0004$), Cd ($p = 0.004$), Pb ($p = 0.002$), and Zn ($p = 0.01$). However, all measured concentrations except Cd and Zn in CG were below US Environmental Protection Agency chronic criteria for the protection of aquatic life. Biotic Ligand Model LC_{50} predictions for Cd and Cu were below our measured concentrations, but measured zinc concentrations exceeded LC_{50} model

Table 1 Dissolved metals concentrations (μg/l) in Gold Creek, Idaho (mean with standard deviation in parentheses)

Parameter	CG	UGC	LGC	Delta	WG
pH	7.1 (0.52)	8.0 (0.07)	8.2 (0.02)	8.3 (0.01)	8.1 (0.06)
Hardness as mg/l $CaCO_3$	19 (3.2)	55 (10)	58 (9)	68 (11)	57 (12)
Arsenic	12 (6.4)*	5.5 (1.4)*	1.2 (0.24)	1.1 (0.40)	0.91 (0.23)
Cadmium	0.12 (0.08)*	0.06 (0.01)*	<0.006	<0.006	<0.006
Copper	<2.5	<2.5	<2.5	<2.5	<2.5
Lead	0.11 (0.06)	0.13 (0.04)*	0.07 (0.03)	0.08 (0.03)	0.04 (0.02)
Zinc	85 (59)*	31 (9.6)*	7.8 (3.0)	10 (4.5)	7.8 (2.6)

* Significant difference compared with the reference site (WG). All tests considered statistically significant at $\alpha \leq 0.05$

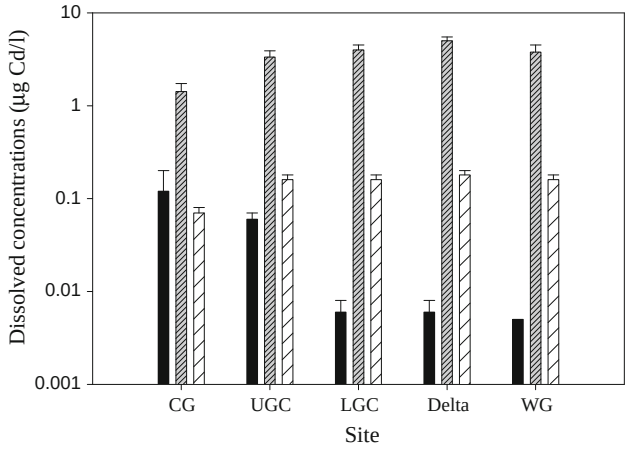

Fig. 2 Dissolved concentrations (µg/l) of measured (*solid*), predicted LC_{50} values (*fine shading*), and US EPA chronic water quality criteria (*coarse shading*) for Cd in Gold Creek, Idaho

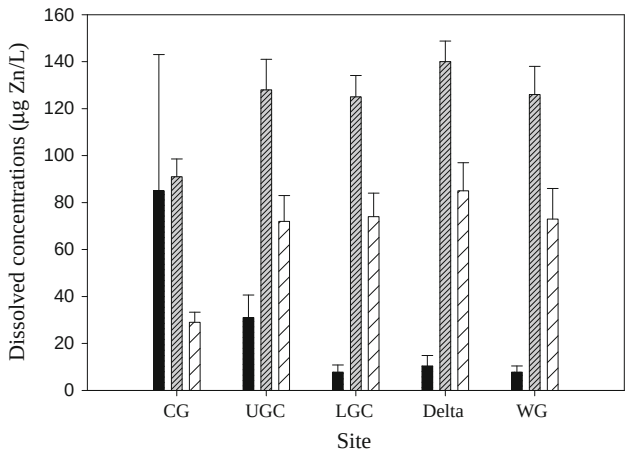

Fig. 3 Dissolved concentrations (µg/l) of measured (*solid*), predicted LC_{50} values (*fine shading*), and US EPA chronic water quality criteria (*coarse shading*) for Zn in Gold Creek, Idaho

predictions in two reaches within CG below Lakeview Mine (Figs. 2, 3).

Concentrations of As ($p < 0.0001$), Cd ($p < 0.0001$), Cu ($p < 0.0001$), Pb ($p < 0.0001$), and Zn ($p < 0.0001$) in sediments (mg/kg) were significantly higher at all sites compared with the reference site (Table 2). In all cases, sediment metals concentrations followed the general trend of CG > UGC > LGC > Delta > WG. Arsenic concentrations in CG were up to 200 times greater than in WG. Arsenic sediment concentrations decreased by 92% from a mean of 537 mg As/kg in CG below Lakeview Mine to a mean of 27 mg As/kg at the Delta over a stream distance of 8.2 km. The graded extent of sediment contamination was clear and followed a consistent downstream decreasing pattern for all analytes. Concentrations of organic contaminants in sediment were low throughout the study area, and below levels thought to impact stream biota.

Benthic Macroinvertebrate Tissues

Concentrations of As ($p < 0.0001$ to $p = 0.001$), Cd ($p < 0.0001$ to $p = 0.01$), Cu ($p < 0.0001$ to $p = 0.04$), Pb ($p < 0.0001$), and Zn ($p < 0.0001$) in benthic macroinvertebrate tissues (µg/g) were significantly higher at all sites compared with the reference site (Table 3). Concentrations in tissues were correlated with those in sediment, and decreased as a function of distance from mining sites. Chloride Gulch had the highest mean benthic macroinvertebrate tissue concentrations for all metals except Pb, which was greater at UGC despite lower Pb sediment concentrations. Mean benthic macroinvertebrate As concentrations were 13 times higher at CG compared with the reference site.

Fish Tissues

Metals concentrations in fish tissues (µg/g) followed a concentration gradient covarying with concentrations found in sediment and benthic macroinvertebrate tissues (Figs. 4, 5). Fish tissue metals concentrations were significantly higher compared with the reference site for all analytes ($p < 0.0001$) in CG and UGC (Table 4). Below Lakeview Mine in CG, fish tissue As concentrations were up to ten times higher than those in the reference site. Concentrations of Cd ($p < 0.0001$), Cu ($p = 0.0007$), Pb ($p = 0.0001$), and Zn ($p = 0.001$) in LGC were significantly higher compared with the reference site. In the Delta, fish tissue metals concentrations were significantly higher for Cd ($p = 0.003$) and Pb ($p = 0.05$) compared with reference site fish tissues.

Table 2 Weak-acid extracted sediment concentrations (mg/kg dry wt.) in Gold Creek, Idaho (mean with standard deviation in parentheses)

Parameter	CG	UGC	LGC	Delta	WG
Arsenic	537 (168)*	50.2 (11.9)*	38.2 (10.4)*	27.7 (12.6)*	2.62 (0.59)
Cadmium	3.56 (1.07)*	2.08 (0.25)*	0.65 (0.19)*	0.57 (0.18)*	0.06 (0.03)
Copper	51.3 (22.4)*	32.3 (7.30)*	9.6 (1.9)*	11.2 (4.6)*	2.78 (1.6)
Lead	304 (97.1)*	249 (67.2)*	56.1 (13.9)*	60.9 (19.1)*	5.1 (1.5)
Zinc	836 (355)*	688 (111)*	232 (54.3)*	247 (41.8)*	7.5 (2.0)

* Significant difference compared with the reference site (WG). All tests considered statistically significant at $\alpha \leq 0.05$

Table 3 Benthic macroinvertebrate tissue metals concentrations (μg/g dry wt.) collected in Gold Creek, Idaho (mean with standard deviation in parentheses)

Parameter	CG	UGC	LGC	Delta	WG
Arsenic	96.7 (35.9)*	41.1 (18.3)*	20.6 (5.10)*	28.2 (1.91)*	5.40 (1.60)
Cadmium	5.81 (0.94)*	3.23 (0.35)*	1.60 (0.14)*	1.68 (0.05)*	1.15 (0.33)
Copper	64.1 (10.1)*	43.9 (2.55)*	23.0 (1.69)*	26.1 (2.26)*	19.5 (2.64)
Lead	41.1 (6.49)*	68.5 (13.2)*	12.5 (2.01)*	21.2 (6.92)*	2.55 (1.34)
Zinc	1870 (431)*	990 (157)*	746 (62.5)*	719 (52.3)*	206 (26.5)

* Significant difference compared with the reference site (WG). All tests considered statistically significant at $\alpha \leq 0.05$

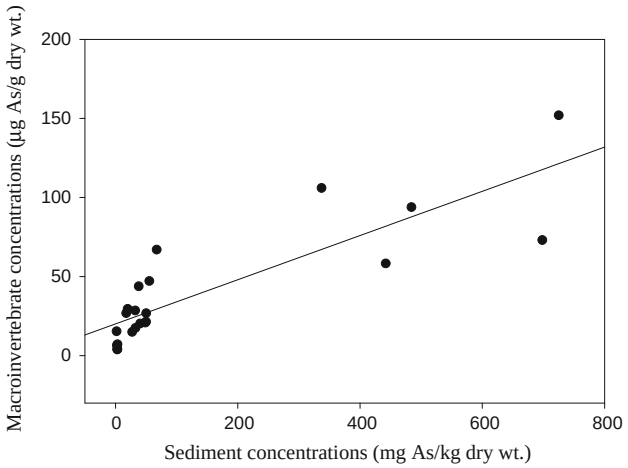

Fig. 4 Scatterplot of relationship between As concentrations in sediment and benthic macroinvertebrate tissues in Gold Creek, Idaho

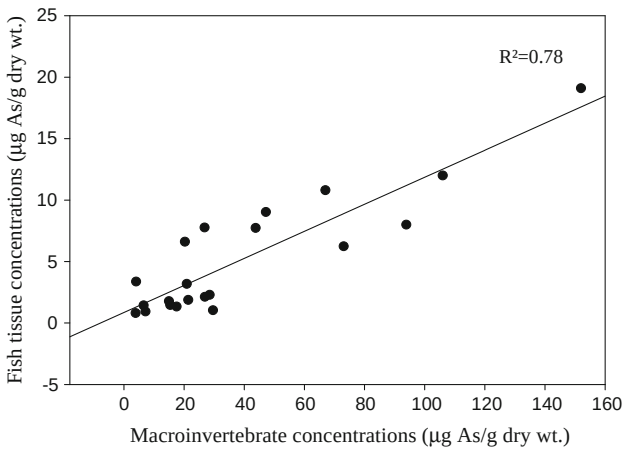

Fig. 5 Scatterplot of relationship between As concentrations in benthic macroinvertebrate tissues and fish whole-body tissues in Gold Creek, Idaho

Histopathology

Livers from westslope cutthroat in CG below Lakeview Mine showed mild glycogen vacuolation and ceroid-like cytoplasmic inclusions, and one fish had moderately enlarged nuclei and individual cell necrosis in hepatocytes. Westslope cutthroat livers in UGC below Conjecture Mine showed moderate glycogen vacuolation and scattered inflammation, and one fish had moderate scattered degeneration of hepatocytes. Bull trout livers in LGC showed moderate nuclear enlargement, scattered degeneration, and necrosis of hepatocytes with scattered foci of inflammation. Bull trout in the Delta had the most numerous and severe liver alterations, including moderate vasculitis in one fish, moderately severe pleomorphism, and moderate degeneration and necrosis in three fish (Table 5; Figs 6, 7).

Discussion

Our observations suggest that bull trout are exposed to and impacted by mining-related metals pollution. Bull trout collected as far as 8 km downstream of source areas had structural liver damage consistent with contaminant exposure, including cell necrosis, degeneration, and pleomorphism. These findings, along with low concentrations of dissolved metals in water and high concentrations of metals in sediment, benthic macroinvertebrates, and fish tissues, suggest that salmonids are exposed to toxic concentrations of metals through a dietary pathway. These results are consistent with other studies in which dietary exposure of metals resulted in cellular damage and reduced health of salmonids (Cockell et al. 1991; Farag et al. 1999, Hansen et al. 2004; Pedlar et al. 2002; Vighi 1981; Woodward et al. 1994). However, to our knowledge, this is the first field study to assess dietary exposure and effects of metals to threatened bull trout.

Aqueous Exposure

Dissolved metals concentrations in water at our sites did not appear to pose a major risk to salmonid health within the study area. All metal concentrations in water, except Cd and Zn, were lower than US Environmental Protection Agency acute and chronic criteria for protection of aquatic life throughout the study area (USEPA 2006). Incorporating the Biotic Ligand Model into our analysis, we were

Table 4 Fish tissue metals concentrations (μg/g dry wt.) collected in Gold Creek, Idaho (mean with standard deviation in parentheses)

Parameter	CG	UGC	LGC	Delta	WG
Arsenic	10.9 (4.96)*	8.38 (1.59)*	2.10 (0.694)	1.61 (0.426)	1.15 (0.33)
Cadmium	1.10 (0.354)*	0.899 (0.236)*	0.473 (0.105)*	0.267 (0.103)*	0.129 (0.0287)
Copper	7.03 (1.89)*	6.31 (0.873)*	4.33 (0.832)*	2.92 (0.428)	2.58 (0.355)
Lead	1.43 (0.684)*	3.10 (2.51)*	1.14 (1.37)*	0.738 (0.382)*	0.112 (0.067)
Zinc	349 (77.4)*	312 (98.1)*	216 (29.7)*	169 (12.5)	128 (10.6)

* Significant difference compared with the reference site (WG). All tests were considered statistically significant at $\alpha \leq 0.05$

Table 5 Histopathological alterations observed in westslope cutthroat (WG, CG, UGC) and bull trout (LGC, Delta) hepatocytes in Gold Creek, Idaho

Site	Number	Severity	Histopathological alteration
WG	5	None to mild	Inflammation
CG	4	Mild	Glycogen vacuolation and ceroid-like inclusions
	1	Moderate	Nuclear enlargement and necrosis
UGC	4	Moderate	Glycogen vacuolation and inflammation
	1	Moderate	Scattered degeneration
LGC	5	Moderate	Nuclear enlargement
			Scattered degeneration/necrosis
			Scattered foci of inflammation
Delta	1	Mild	Glycogen vacuolation and vasculitis
	1	Mild/moderate	Glycogen vacuolation and pleomorphism/degeneration
	2	Moderate/severe	Degeneration/necrosis and pleomorphism
	1	Moderate/severe	Pleomorphism and degeneration/necrosis and inflammation

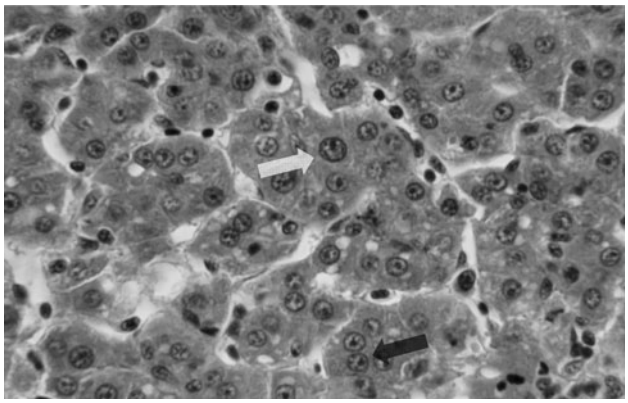

Fig. 6 Illustration of bull trout hepatocyte nuclei pleomorphisms from Gold Creek, Idaho. *Light arrow* indicates single nuclear pleomorphism and *dark arrow* indicates normal hepatocyte nuclei

able to predict rainbow trout LC_{50} values (concentration causing 50% mortality) for Cd, Cu, and Zn based on measured stream chemical parameters. Model predictions for LC50 concentrations were much higher than our measured metals concentrations in water for all metals, with the exception of Zn directly below Lakeview Mine.

Dietary Exposure

Sediment metals concentrations measured in the Gold Creek drainage may be high enough to reduce health of native salmonids, including bull trout within Gold Creek. The sediment metals concentrations measured in CG and UGC were similar to sediment metals concentrations in some areas of the Clark Fork and Coeur d'Alene Rivers of Montana and Idaho. These rivers have extensive mining-related metals contamination, and the adverse effects on biota were well documented (Farag et al. 1995, 1999; Woodward et al. 1994). However, sediment concentrations of As in Chloride Gulch sediments are higher than those measured in most areas of these other river systems (Farag et al. 1998; Hansen et al. 2004). Researchers have noted that As uptake through the dietary pathway results in elevated fish tissue metals burdens, and characteristic physical effects are highly correlated with sediment concentrations (Farag et al. 2007; Hansen et al. 2004).

Macroinvertebrates within Gold Creek accumulated sediment-derived metals and may present a potential risk of diet-borne exposure of metals to salmonids. Several studies

have noted the dietary effects to salmonids from elevated metals within benthic macroinvertebrate tissues. Farag et al. (1999) used benthic macroinvertebrates collected from the Coeur d'Alene River and fed them to cutthroat trout in a laboratory study. The metals concentrations within the diet were 13.5 μg As/g, 29.1 μg Cd/g, 43.8 μg Cu/g, 452 μg Pb/g, and 2,119 μg Zn/g. Although the levels of Cd and Pb were greater in this diet compared with our field-collected benthic macroinvertebrates, levels of Cu and Zn were similar, and As concentrations within Gold Creek benthic macroinvertebrate tissues were up to eight times greater. The Coeur d'Alene River study found that metals incorporated into benthic macroinvertebrate tissues efficiently transferred across the gut and resulted in elevated metals within fish tissues. The diet resulted in reduced survival, growth, and histopathological alterations to fish tissues (Farag et al. 1999). The results of our study are concordant with laboratory studies in which transfer of metals through the dietary pathway resulted in deleterious effects to salmonids, including increased metals tissue burdens resulting in destructive cellular alterations within the liver (Hansen et al. 2004; Vighi 1981; Woodward et al. 1994).

Pathway

Metals distributions in various physical and biological ecosystem pools showed a clear and consistent pattern throughout the study area. Metals concentrations in benthic macroinvertebrates and fish were proportional to concentrations found in sediments. Generally, the concentration gradient followed the trend sediments > benthic macroinvertebrates > fish > water for As, Cu, and Pb, and benthic macroinvertebrates > sediment > fish > water for Cd and Zn (Tables 1–4). Metals concentrations were greatest below mine sites and decreased downstream to the Gold Creek Delta. However, the ratio of metals within sediments and biotic tissues increased further downstream of mine sites, indicating that metals were more bioavailable at downstream sites. For example, As concentrations were nearly equal in benthic macroinvertebrate tissues and sediments at LGC and the Delta.

Regression analysis suggests that all metals were transferred to salmonids through the dietary pathway, but As had the strongest correlations (Table 6). Although water and fish tissue As concentrations were significantly correlated ($R^2 = 0.55$) they were weaker than food-chain pathways ($R^2 = 0.78$). This research corroborates other studies that have found strong correlations between benthic macroinvertebrate and fish tissue As concentrations (Farag et al. 2007; Hansen et al. 2004). These findings along with others suggest that the dietary pathway may be more predominant for As compared with other toxic metals.

Field Observations

Fish below mine sites showed symptoms indicative of metal exposure including large distended stomachs and

Table 6 Regression analysis of contaminant pathways to biological receptors in Gold Creek, Idaho

Analyte	Independent	Dependent	Equation	*T*-value	R^2 (adj.) (%)
Arsenic	Water	Benthic tissues	$y = 15.6 + 5.22x$	5.34	56
	Water	Fish tissues	$y = 2.08 + 0.66x$	5.44	55
	Sediment	Benthic tissues	$y = -63.5 + 5.17x$	7.22	70
	Benthic tissues	Fish tissues	$y = 0.86 + 0.11x$	8.68	78
Cadmium	Water	Benthic tissues	$y = 1.88 + 21.2x$	4.22	44
	Water	Fish tissues	$y = 0.33 + 5.04x$	5.46	55
	Sediment	Benthic tissues	$y = 1.04 + 1.21x$	11.85	86
	Benthic tissues	Fish tissues	$y = 0.13 + 0.16x$	4.28	46
Copper	Water	Benthic tissues	N/A	N/A	N/A
	Water	Fish tissues	N/A	N/A	N/A
	Sediment	Benthic tissues	$y = 20.5 + 0.71x$	7.13	70
	Benthic tissues	Fish tissues	$y = 1.36 + 0.09x$	6.93	70
Lead	Water	Benthic tissues	$y = -3.35 + 381x$	5.09	54
	Water	Fish tissues	$y = 0.39 + 9.80x$	1.53	5
	Sediment	Benthic tissues	$y = 9.61 + 0.14x$	5.04	53
	Benthic tissues	Fish tissues	$y = 0.41 + 0.03x$	2.51	21
Zinc	Water	Benthic tissues	$y = 673 + 8.32x$	2.93	26
	Water	Fish tissues	$y = 180 + 1.71x$	4.48	45
	Sediment	Benthic tissues	$y = 314 + 1.47x$	8.05	75
	Benthic tissues	Fish tissues	$y = 145 + 0.10x$	3.07	29

pronounced darkened skin coloration. Woodward et al. (1994) observed that more than 50% of brown trout (*Salmo trutta*) fed benthic macroinvertebrates from the Clark Fork River, MT exhibited swollen abdomens, and constipation. The authors suggested that the swollen stomachs were a result of gut impaction, and attributed the observed physiological effects to exposure of dietary metals. Arsenic has been shown to induce melanin production, and increased chromatophores may be suitable biomarkers of As toxicity (Allen et al. 2004). Farag et al. (2003) observed that fish held in cages in the Boulder River, MT below mine sites with elevated As concentrations developed darkened skin coloration.

Fish Tissue Metals Burdens

Westslope cutthroat and bull trout are accumulating metals within their tissues in Gold Creek, and concentrations may be high enough to compromise fish health. This study found a dry weight average in fish tissues of 10.9 μg As/g, 1.1 μg Cd/g, 7 μg Cu/g, 1.4 μg Pb/g, and 349 μg/g in CG and 8.3 μg As/g, 0.89 μg Cd/g, 6.3 μg Cu/g, 3.1 μg Pb/g, and 312 μg Zn/g in UGC, which are consistent with other studies where similar fish tissue metals burdens resulted in deleterious effects to salmonids (Woodward et al. 1994; Farag et al. 1999). However, As concentrations in this study were higher than those measured in the previous two studies (Table 4). Farag et al. (2003) measured 8 μg As/g dry weight whole-body concentrations in rainbow trout (*Onchorynchus mykiss*) in the Boulder River (MT) below mine sites. These were the highest whole-body As concentrations observed within the entire river drainage. Whole-body burdens were highly correlated with sediment and thought to have been derived primarily through dietary exposure. Although other metals were elevated within tissues, these body burdens led to decreased health, including increased metallothionein, lipid peroxidation, reduced growth, and depressed fish densities.

Histopathological Response

The histopathological alterations we observed were consistent with other findings where dietary exposure to metals resulted in cellular damage. Pedlar et al. (2002) fed lake whitefish a diet of 1–100 μg As/g and observed nuclear and architectural alterations to livers. Woodward et al. (1994) observed nuclear pleomorphism, cytoplasmic vacuolation, and scattered degenerate hepatocytes in rainbow trout fed a metals-contaminated diet with concentrations in benthic macroinvertebrates similar to those measured in our study. Many studies have noted the liver as the primary site of toxic action for metals in fish (Lage et al. 2006). We observed histological aberrations in other tissues, but hepatic tissues were the most consistent and damaged tissues. Our study supports previous work in which liver histopathological alterations could serve as a useful biomarker of dietary metals exposure and effects. Although structural liver alterations were observed in westslope cutthroat with higher metals concentrations in tissues, liver alterations were more numerous and severe in bull trout with lower tissue metals concentrations. The original study plan sought to collect age-0 bull trout throughout the study area and collect age-0 westslope cutthroat as surrogates where bull trout were absent. We were unable to collect age-0 salmonids in CG or UGC, and therefore collected age-1 westslope cutthroat. The mean length of westslope cutthroat and bull trout collected for histology samples was 70 and 56 mm, respectively. This size and age difference could have affected the degree of histopathological abnormalities we observed (Bernet et al. 1999). It is well documented that young juvenile fish are more susceptible to metals exposure than are larger, older fish (Buhl and Hamilton 1990; Chapman 1978; Eaton et al. 1978). Older fish with history of exposure may develop mechanisms for more efficient metal excretion, thus limiting cellular damage (Lage et al. 2006). Alternatively, the most sensitive individuals may have been lethally removed from the population, and we were only able to collect the most fit, most resistant individuals that demonstrated less severe effects. Lastly, bull trout may be more susceptible to metals due to physiological differences between the two species. It is well known that species can have vastly different tolerances to metals insults (Buhl and Hamilton 1990; Luoma and Rainbow 2005). These interspecies differences may account for the degree of histopathological alterations we observed between groups.

It is unlikely that fish movements contributed to any differences we observed in histopathological alterations between sites due to temporal hydrological conditions. During summer and early fall the middle section of Gold

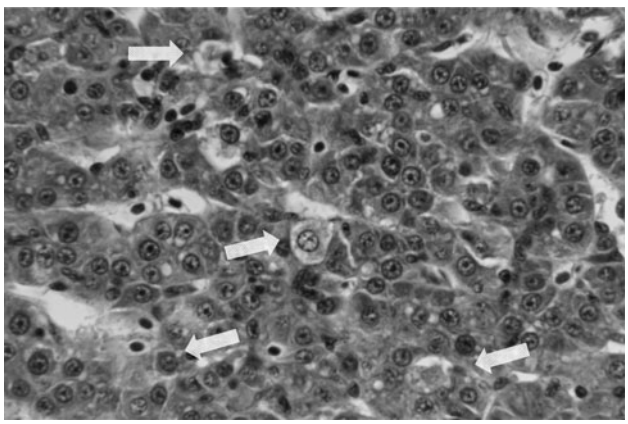

Fig. 7 Illustration of scattered degeneration and single-cell necrosis (*light arrows*) of bull trout hepatocytes from Gold Creek, Idaho

Creek flows subsurface, preventing spawning bull trout from reaching upstream sections below mine sites. Therefore, young-of-year bull trout we captured in LGC and the Delta had likely originated within the primary spawning areas within LGC. Other studies within the Lake Pend Oreille Basin have shown that young-of-year bull trout generally make downstream movements and would not have been able to reach upstream areas below mine sites during high spring flows (Downs et al. 2003).

Several laboratory studies have noted gallbladder lesions in salmonids associated with dietary arsenic, and suggested that gallbladder pathology may be a useful indicator of arsenic exposure in field studies (Pedlar et al. 2002). Interestingly, we did not observe any of these characteristic lesions in our samples despite high As concentrations in benthic macroinvertebrate prey items. However, most laboratory studies are less than 90 days in duration, and those studies documented initial acute stages of gallbladder pathology (Cockell and Bettger 1993; Cockell and Hilton 1988; Cockell et al. 1991). Individuals that are more susceptible may develop these characteristic lesions and be removed from the population at critical early life stages, thus making it difficult to document in field studies from surviving individuals. Our findings suggest that gallbladder pathology may not be a useful indicator of chronic dietary arsenic exposure under complex field conditions.

Arsenic speciation may also play a major role in affecting toxicological responses to aquatic organisms. Frankenberger (2001) suggests that, to assess risk from arsenic to aquatic biota, arsenic speciation must be taken into account. Differing forms and species of arsenic can have vastly differing toxicological results on organisms (Lage et al. 2006). However, determining arsenic species in sediments and tissues has proven challenging. Jankong et al. (2007) reported extraction efficiencies for As in fish tissues as low as 60% in snakehead (*Channa argus*) fish tissues. Furthermore, little is known about the varying effects of each As species to fish in general, and salmonids specifically. Arsenic speciation could have explained some of the variation we observed in histopathological endpoints between sites. Further research on arsenic speciation and toxicological effects in fish is warranted.

Field Versus Laboratory Studies

Laboratory studies may underestimate effects of diet-borne contaminants to salmonids. Other research suggests that, in laboratory exposures, the diets may be different from natural food items. Some laboratory study diets were supplemented with vitamins and minerals, thus potentially modifying the diet from what would normally be consumed (Woodward et al. 1994). Other studies exposed live diets to metals for brief or limited exposure times (Hansen et al. 2004). Under field conditions, metals may be bound to tissues in prey items quite differently from in laboratory food items (Farag et al. 1999). For example, benthic macroinvertebrates exposed to metals for their entire life cycle may incorporate metals within internal tissues differently from during short-term laboratory exposures. These differences could have vastly different toxicological effects, and natural diet-borne exposure may be more toxic than laboratory diets (Woodward et al. 1994). Furthermore, laboratory studies are conducted in relatively benign environments. Gauthier et al. (2006) found that fathead minnows (*Pimephales promelas*) under field conditions showed a biological response to low levels of aqueous Cd and Ni, including prolonged hatching time and increased mortality, whereas no effects were observed in laboratory counterparts. The authors attributed the differences to higher stress associated with natural environments which may sensitize fish, thus making fish more susceptible to the toxic effects of contaminants under stressful field conditions. Additional insults from metals exposure may greatly reduce young salmonids' ability to compete for food and cover, elude predators, and cope with additional stressors (e.g., increasing thermal regimes). Therefore, site-specific field investigations can be invaluable in evaluating risks to threatened species such as bull trout.

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